HUDSON INSTITUTE TO PRESENT PHOTOSOFT™ AT PRESTIGIOUS COMBIO2022 INDUSTRY CONFERENCE

MELBOURNE (AUSTRALIA) 27 September 2022: Invion Limited (ASX: IVX) ("**Invion**" or the "**Company**") wishes to announce that Hudon Institute of Medical Research (**Hudson Institute**) is presenting a poster on the Photosoft™ technology at the prestigious ComBio2022 industry conference to be held in Melbourne from 27-30th September 2022.

ComBio is the Australian Society for Biochemistry and Molecular Biology's (ASBMB's) national biennial conference, which runs in conjunction with other societies and features world renowned international speakers.

The poster presentation by Hudson Institute (attached to the end of this announcement) compares a Photosoft™ compound with two other chlorin-based photosensitisers including Talaporfin sodium, which is approved in Japan for the treatment of lung cancer.

The key findings highlighted in the poster found that phyllochlorin sodium – a compound that belongs to a new group of chlorin-based photosensitisers within the Photosoft™ technology suite –– demonstrated the following *in vitro* and *in vivo*:

- Exhibited rapid cellular uptake and clearance in ovarian cancer lines
- Strongly localised to endoplasmic reticulum (ER) upon uptake into cells
- Accumulated rapidly and was retained in tumour tissue for at least 48 hours when injected *in vivo* (syngeneic mouse model of triple negative breast cancer)
- Immediately and efficiently produced reactive oxygen species (ROS) when exposed to light at 660nm, and thereby cell death in the tumour
- Showed low dark toxicity in the absence of light activation and significantly greater phototoxicity upon illumination when compared with the other two compounds

Hudson Institute's research offers insight into the development of a novel family of photosensitisers based on the Photosoft™ technology and their potential clinical efficacy and application for Photodynamic Therapies.

This announcement was approved for release by Thian Chew, Chairman of the Board.

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About Invion

Invion is a life-science company that is leading the global research and development of the Photosoft™ technology for the treatment of a range of cancers, atherosclerosis and infectious

diseases. Invion holds the exclusive Australia and New Zealand license rights and exclusive distribution rights to Asia Pacific excluding China (other than Hong Kong, which is included in the Territory), Macau, Taiwan, Japan and South Korea to the Photosoft™ technology for all cancer indications. It also holds the exclusive rights to the technology in Asia Pacific (excluding Greater China) for atherosclerosis and infectious diseases. Research and clinical cancer trials are funded by the technology licensor, RMW Cho Group Limited, via an R&D services agreement with the Company. Invion is listed on the ASX (ASX: IVX).

About Photodynamic Therapy (PDT)

Invion is developing Photosoft™ technology as a novel next generation Photodynamic Therapy (PDT). PDT uses non-toxic photosensitisers and light to selectively kill cancer cells and promote an anti-cancer immune response. Less invasive than surgery and with minimal side effects, PDT offers an alternative treatment option aimed at achieving complete tumour regression and long-lasting remission.

Identification and characterisation of Phyllochlorin sodium, a novel chlorin-derived photosensitiser

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Background:

Photodynamic therapy (PDT), combining a photosensitising compound and light to generate destructive reactive oxygen species (ROS), is an effective strategy for tumour tissue ablation. FDA-approved for the treatment of several cancer types, a lack of efficacy and undesirable toxic side effects associated with many existing photosensitisers has largely restricted their use to superficial tumours. This work presents data characterising a new chlorin-based photosensitiser - Phyllochlorin sodium - *in vitro* and *in vivo*.

Methods:

Phyllochlorin sodium absorbance and fluorescence spectra and reactive oxygen species (ROS) production on illumination at 660 nm were compared with two photosensitisers of Chlorin family, Chlorin e4 sodium and the clinically approved Talaporfin sodium (Laserphyrin®, mono-L-aspartyl chlorin e6) in a cell-free system. *In vitro* cellular uptake and clearance were monitored using fluorescence in ovarian cancer cell lines, with subcellular localisation determined using confocal microscopy with specific organelle staining for endoplasmic reticulum (ER), mitochondria and lysosome. Cell viability with (for phototoxicity) and without (for dark-toxicity) exposure to red light (660 nm) was determined by AlamarBlue assay. Mice with established primary breast tumours received Phyllochlorin sodium by intravenous (IV) injection, oral gavage, or intratumoral (IT) injection. At pre-determined intervals post-administration, the localisation of Phyllochlorin sodium in tumours was quantified using the IVIS Lumina Imaging System to provide the average radiant efficiency as a measure of fluorescence of the tumour area at each time point.

Results:

Phyllochlorin sodium shares spectral characteristics to other chlorin-derived photosensitisers.

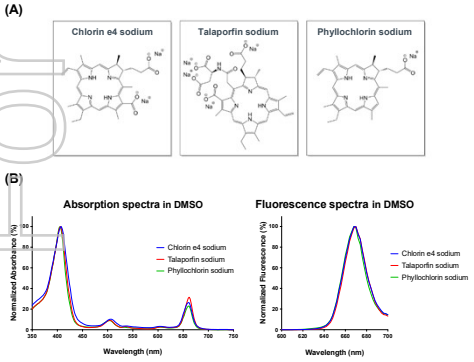
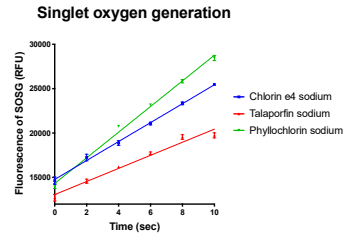


Figure 1. (A) Chemical structure of Chlorin e4 sodium, Talaporfin sodium and Phyllochlorin sodium. **(B)** Absorption and fluorescence spectra of Chlorin e4 sodium, Talaporfin sodium and Phyllochlorin sodium in DMSO. Absorbance was scanned 350-750 nm in 2 nm increments. Fluorescence emission with excitation at 400 nm was scanned 600-700 nm in 2 nm increments.

Phyllochlorin sodium shows enhanced rate of singlet oxygen production than other chlorin-derived photosensitisers.



	Chlorin e4 sodium	Talaporfin sodium	Phyllochlorin sodium
Best-fit values			
Slope	1066	736.7	1445

Figure 2. Upon illumination at 660 nm, time course of formation of singlet oxygen as determined by measuring the fluorescence of the fluorescent probe Singlet Oxygen Sensor Green (SOSG). The slope of the linear regression curve represents the rate of the singlet oxygen generation reaction. n=4.

Phyllochlorin sodium accumulates and releases rapidly in cancer cells *in vitro*.

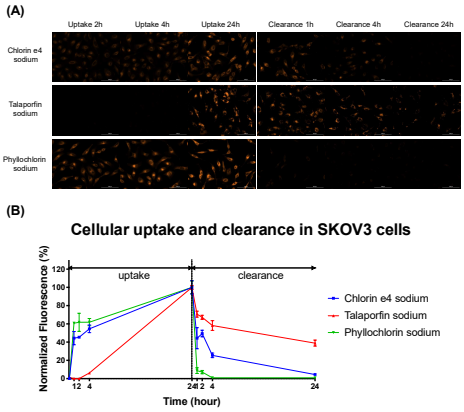


Figure 2. (A) Cellular uptake and clearance of Phyllochlorin sodium, Chlorin e4 sodium and Talaporfin sodium in SKOV3 ovarian cancer cells over time. Images were captured and analysed using the Cytation 3 Multimode Imager. **(B)** Fluorescence intensity was generated from images, and data were expressed as a percentage of maximum (100%) fluorescence which was defined at 24 h uptake. n=3.

Upon uptake into cells, Phyllochlorin sodium strongly co-localises to the ER.

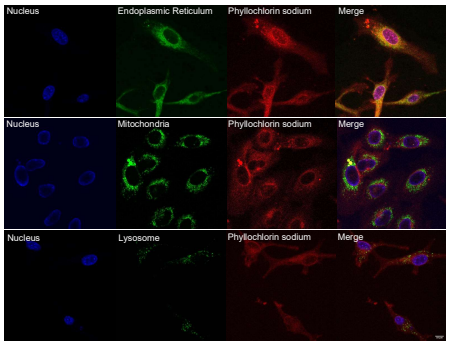


Figure 3. Subcellular localisation of Phyllochlorin sodium in SKOV3 ovarian cancer cells. Cells treated with Phyllochlorin sodium were stained with ER-Tracker Green, MitoTracker Green or LysoTracker Green and cell nuclei counterstained with Hoechst 33342. Images were captured using a 100x oil objective lens on confocal microscope. Scale bars: 10 µm.

Phyllochlorin sodium demonstrates low dark toxicity and greater phototoxicity than other chlorin photosensitisers.

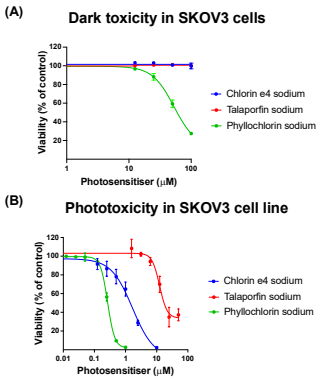


Figure 6. (A) Dark toxicity (without light activation) and **(B)** phototoxicity (exposure to red light of 660 nm) of Phyllochlorin sodium, Chlorin e4 sodium and Talaporfin sodium in SKOV3 ovarian cancer cells was assessed using AlamarBlue assay. Viability was expressed as % of untreated controls. n=4.

Phyllochlorin sodium localises to tumours *in vivo*.

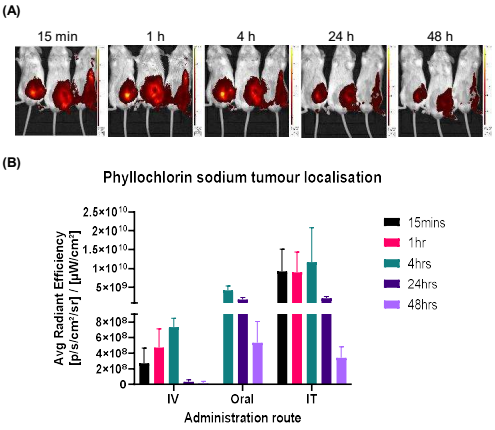


Figure 4. Localisation of Phyllochlorin sodium in tumours. (A) Mice bearing breast tumours received Phyllochlorin sodium 1mg/kg by intravenous (IV), oral gavage or intratumoural (IT) administration routes. After 15 min, 1 h, 4 h, 24 h and 48 h, Phyllochlorin sodium fluorescence in tumour was imaged using the IVIS Lumina *in vivo* Imaging System. **(B)** Quantitative average radiant efficiency of the tumour area was calculated and corrected for the vehicle control. n=3/group.

Conclusions:

Compared to other Chlorin photosensitisers, Phyllochlorin sodium demonstrated:

- Similar spectral properties;
- Substantially enhanced ROS production and phototoxicity, with an increased therapeutic window;
- Excellent localisation and retention to tumour tissue *in vivo*.

Our study offers insight into the development of a novel family of photosensitisers and their potential clinical efficacy and application for PDT.

Acknowledgments:

We acknowledge Invion Limited and RMW Cho Group.